COMPARISON OF THE CHROMATOPHOROTROPIC ACTIVITY OF INSECT CORPORA CARDIACA WITH THAT OF CRUSTACEAN SINUS GLANDS[1]

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For a number of years it has been suspected from their histology that the corpora allata and the corpora cardiaca of insects have endocrine functions. Finally, in 1934, Wigglesworth demonstrated that tissues in the head of *Rhodnius* were responsible for humoral substances which were concerned with molt and metamorphosis, and later, in 1936, that growth and reproduction were also controlled to some extent by hormones. The corpora allata were suspected to be the source of the hormones concerned. Fraenkel (1935) demonstrated that a substance arising in the head of certain fly larvae was responsible for pupation in these forms. Pfeiffer (1939), by extirpation experiments, has shown that the gland concerned in the control of the activity of certain cells of the oviduct of the grasshopper was definitely the corpus allatum, as had been strongly suspected to be the gland concerned with the functions investigated both by Wigglesworth and by Fraenkel. Hanström (1936, 1937, and 1938) in a series of papers reported that the heads of insects, like the eyestalks of crustaceans, secreted a substance highly active in modifying the state of concentration and dispersion of crustacean chromatophores. In crustaceans Hanström had presented good support of the hypothesis that the sinus gland of the crustacean eyestalk was the source of the chromatophorotropic material. He was, however, unable to conclude just what was the source of the apparently comparable material from the insect head.

The greatest part of the work upon the insect endocrine glands has concerned the corpora allata. As yet no definite function has been ascribed to the corpora cardiaca. Recent work of Pfeiffer (1939) has given her some reason for suspecting that at least in *Melanoplus*, the corpora cardiaca or some structure located in the same region might be more directly concerned with molting in this form than are the corpora allata.

In the light of the work by Brown and Cunningham (1939) which presented evidence that the sinus gland of *Cambarus* was directly concerned with control of molting in this form, and that of Brown (1940) showing that the sinus gland is also the eyestalk gland which produces chromatophorotropic hormones, it became interesting to discover whether in the insect as well, the same two functions (molt control and chromatophore activation) were to be found in a single gland. Were this true, then it would be the corpora allata to be first suspected since these were most generally believed to be the organs of the insect concerned with development.

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Experimental

For these experiments the cockroach, *Periplaneta americana,* was used as the source of the insect tissues and the crayfish, *Cambarus immunis,* was the source of the crustacean tissues. After trying a number of methods of testing the action of various tissue extracts of the insect body upon crustacean chromatophores, it was finally decided to use isolated chromatophores as the test object. This practice was chosen inasmuch as it was possible to obtain great uniformity and sensitivity of response. The technic consisted of removing the carapace of a small crayfish (carapace length between 1.6 and 2.5 cm.) in the following manner: with fine scissors the carapace was cut from right to left just anterior to the cervical groove and then the scissors were inserted under the carapace in the median line at the posterior edge of the carapace in such a manner that the latter could be cut by a single stroke of the scissors into right and left halves. Each half was then removed by grasping the ventro-lateral edges of the carapace with forceps and lifting upwards, carefully tearing away the attaching muscles. Removing the carapace halves by grasping the dorsal cut edges invariably ruined the pieces. These two carapace halves, concave side uppermost, were placed in Syracuse watch-glasses, in sufficient Griffeth's solution to cover them. By rocking a sharp, round-pointed scalpel blade over them it was possible with a little practice to cut them up into a number of rectangular pieces (approximately 1 × 4 mm.) under a dissecting microscope. When viewed under a strong beam of light in an opaque white depression slide in a small quantity of Griffeth's solution with a compound microscope, it was possible to distinguish readily two types of chromatophores, red and white. These chromatophores were quite responsive to changes in the constitution of the bathing medium and retained this reactiveness for a number of hours. Furthermore, it was possible, by appropriate treatment of the crayfish which was to be the donor of the carapace, to obtain these two chromatophore types in almost any state of pigment dispersion or concentration. The sensitiveness of the isolated chromatophores as test objects will become apparent in the experiments to be described.

Two difficulties arose in the use of these fragments of carapace in quantitative work. The first was that all of the chromatophores, either red or white, were not physiologically just alike. In the case of the darker red chromatophores there were those that were very sensitive to the dispersing and concentrating influences, and scattered in among these were smaller and paler red chromatophores which were somewhat less responsive. There appeared to be almost a complete series of grades of responsiveness. With the white chromatophores the situation was a little simpler since the few white chromatophores which were physiologically different from the majority lay in distinct patches. These patches were readily distinguishable since it was noted that these lay in the areas relatively free from red chromatophores and towards the dorsal portion of the carapace. By the proper selection of source of the test areas, these patches could be fairly well avoided. The second difficulty lay in the fact that at the greater dilutions of extracts tested, the chromatophores which were affected were more and more restricted to the peripheral region of the rectangular fragments, as if the material were diffusing inward from the cut edge.

In testing an extract sample, about 0.4 cc. of the sample was placed in a glazed porcelain depression and into this was placed one of the small carapace fragments. The general state of the red and white chromatophores was recorded at the beginning of the test and every fifteen minutes thereafter up to an hour. A control piece of carapace in Griffeth's solution was always kept for comparison. The state of the chromatophores was recorded in terms of a chromatophore index in which the chromatophore state at the time of any observation was expressed by a number ranging from one to five depending upon the degree of dispersion. A fully dispersed pigment mass was designated as 5 and a completely concentrated one as 1, with 2, 3, and 4 describing intermediate conditions. Due to the physiological differences of the chromatophores in a piece of integument, it was usually necessary to record a number describing the most fully dispersed chromatophores and another describing the most completely concentrated ones and then take the average value as the response to the extract tested.

The cockroaches used in the following experiments were all rather uniform in size ranging from 3.2 to 3.5 cm. in length (overall, excluding appendages). The crayfishes which served as donors of sinus glands varied in carapace length from 2.8 to 3.4 cm.

The Source of the Chromatophorotropic Hormones in the Insect Head

The first experiments were directed towards a localization of the source of the chromatophorotropic substance responsible for the results obtained by Hanström (1938). For this experiment the following tissues were carefully dissected out of the body of the cockroach: (1) brain; (2) frontal ganglion; (3) each of the three thoracic ganglia; (4) corpora cardiaca; (5) corpora allata. (Figure 1 shows a camera lucida outline of corpora allata and corpora cardiaca after dissection.) Each of the tissues was placed in a small porcelain depression. The brain was triturated and extracted in 0.8 cc. of Griffeth's solution; a single thoracic ganglion, the frontal ganglion, a single corpus allatum, and a single corpus cardiacum were each macerated and extracted in 0.4 cc. of Griffeth's solution. A piece of *Cambarus* carapace was added to each. Two Griffeth's solution controls were kept with each such experimental series. This procedure was repeated five times with the average results found in Fig. 2. The thoracic ganglia have been averaged together since there was not any perceptible difference found in the extracts of the three of them. It is noted that the only tissue of those tested which effected a concentration of the red pigment was the corpus cardiacum. On the other hand, it would appear from the data that brain and frontal ganglia were producing a concentration of the white pigment and that the thoracic ganglia were not doing likewise. Thus the concentrating agent of the brain and the frontal ganglion appears not to be a principle found in nervous tissue generally.

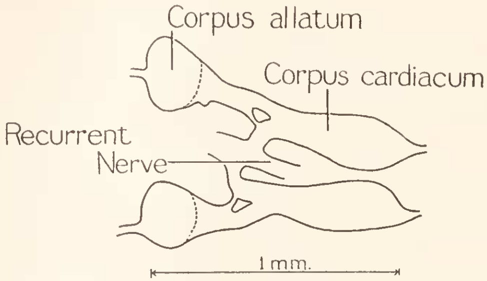

FIG. 1. A camera lucida outline of the isolated corpora allata and corpora cardiaca of the cockroach as seen from dorsal view.

Brief experiments indicated that corpora cardiaca tissue extract was still strongly effective after boiling, and that the gland could be desiccated and stored for 24 hours without losing its capacity to concentrate red pigment when extracted.

The Size of the Cockroach Corpus Cardiacum

The cockroach corpus cardiacum was dissected out and outlined with the aid of a camera lucida. A rough approximation of its volume was made by constructing a clay replica of it and then reforming the clay into a sphere the volume of which was readily calculated from the diameter. Knowing the magnification of the model, it was easily found that the volume of a single gland of the cockroach was about 0.019 mm.^{3}. The limits of the gland in making these measurements were determined by the extent of the bluish cast possessed by the gland when viewed in an intense beam of light. The glandular tissue appears more bluish than the neighboring nervous tissue and somewhat less transparent. In this respect, this gland resembles in appearance the sinus gland of the crayfish and other crustaceans.

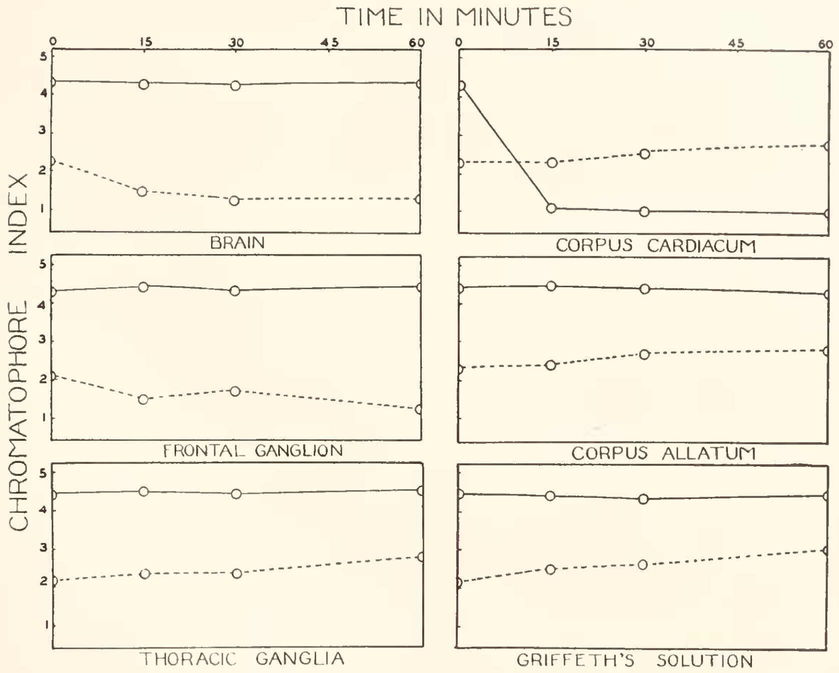

FIG. 2. The responses of red (solid line) and white (broken line) chromatophores of isolated pieces of crayfish integument to extracts of five organs of the cockroach body and to Griffeth's solution. A decreasing index with time indicates concentration of pigment and an increasing one, dispersion.

On the basis of this rough determination it appeared that the corpus cardiacum of our cockroaches was about half the volume of the crayfish sinus gland (Brown, 1940).

The Activities of Cockroach Corpora Cardiaca and Crayfish Sinus Glands as Seen in a Dilution Series

A long series of dilutions was made of each of the corpora cardiaca and the sinus glands and the effect of each member of the series was assayed in the usual manner upon isolated chromatophores. With such a method it was possible to discover the relative differences in the potency of the two tissues and at the same time to distinguish qualitative from quantitative differences in their activities.

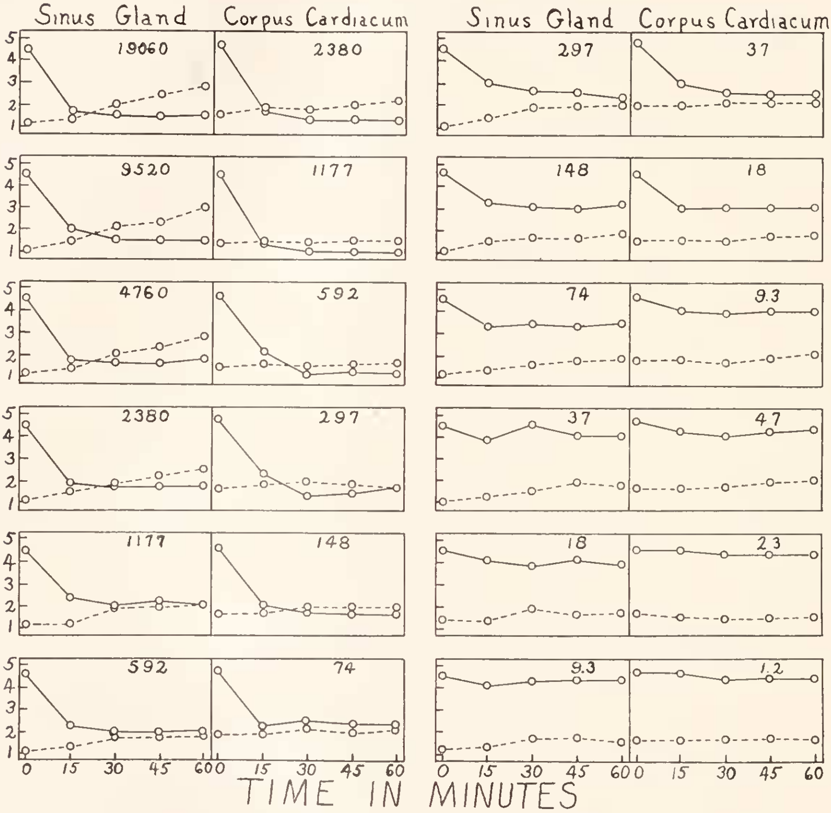

FIG. 3. The effect of dilution of the extracts of the insect corpora cardiaca and crustacean sinus glands upon red and white chromatophores. Red chromatophore responses are indicated by solid lines and white, by broken lines. The numerals along the ordinates are the average chromatophore indices. The numbers enclosed within the frames give the number of units of gland (by volume) to 2×10^8 units of solvent.

For this experiment the corpora cardiaca were removed, permitted to dry briefly in a small mortar and then were triturated thoroughly in 0.8 cc. of Griffeth's solution. Half of this was used as the first sample to be tested and then the remainder was diluted to half the concentration and then half of this latter was set aside for testing. This procedure was repeated seventeen times, or in other words until the concentration was one part (by volume) of gland tissue to 2,760,000,000 parts of Griffeth's solution. It is important to point out that the dilutions were not made in the same vessel in which the glands were originally ground. Thus the results could not be influenced by a continued extraction of the original macerated tissue which might have adhered to the ground glass bottom of the mortar.

Exactly the same procedure was applied to the sinus glands of a crayfish. Two glands were originally extracted in 0.8 cc. of Griffeth's solution and diluted in steps with the concentration of each step being half that of the preceding one. Again here, seventeen such dilutions were made. Calculating on the basis that the volume of the sinus gland is about double that of the corpora cardiaca in animals of the sizes used, the maximum dilution obtained was one part gland to 1,380,000,000 parts of Griffeth's solution.

The above two experiments were repeated five times each. The greatest dilutions effective upon the red pigment for the experiments in which the corpora cardiaca were used were one part gland tissue by volume to:

11,050,000 parts Griffeth's solution
172,000,000 “ “ “
43,000,000 “ “ “
172,000,000 “ “ “
43,000,000 “ “ “

The average of these values is 88,200,000 parts of Griffeth's solution. In contrast to these, the last effective dilutions of the sinus glands fell at one part gland tissue to:

5,400,000 parts Griffeth's solution
2,700,000 “ “ “
43,000,000 “ “ “
1,347,000 “ “ “
21,600,000 “ “ “

The average of these values is 14,810,000 parts of Griffeth's solution. From this one would conclude that if the red pigment-concentrating substance is the same in the two glands the corpus cardiacum tissue contains roughly six times as much active substance per unit volume as the sinus gland.

Figure 3 shows the comparative effects of dilutions of the corpora cardiaca and sinus glands upon the red and white chromatophores. A comparison of these two series yields some very interesting conclusions. (1) There is no significant effect of the extract of the corpora cardiaca upon the white chromatophores, while an extract of the sinus gland definitely dispersed the white pigment. (2) Volume for volume the corpora cardiaca tissue appears to be about eight times as effective upon the red chromatophores as the sinus gland.

DISCUSSION

One interesting thing that these experiments indicate is that the crutacean sinus gland produces a concentration of the red pigment and simultaneously induces a dispersion of the white. At first this would appear to be a contradiction to the findings of Brown (1935) that eyestalk extract caused a concentration of the white when injected into eyestalkless *Palaemonetes*. The explanation of the difference is undoubtedly to be found in the great difference in the amount of eyestalk tissue involved in these two instances. Extracts of many crustacean central nervous organs is effective in concentrating white pigment.

Extracts of the central nervous system[2] of the crayfish are very effective in producing a concentration of both the red and the white pigment. In this we see a qualitative difference between the sinus gland and the nervous system since the former dispersed the white pigment. Although all parts of the crustacean nervous system are more or less effective upon both red and white pigment, the findings in this research indicate that the cockroach does not possess a red concentrating principle in any part of its brain or thoracic nerve cord or in its frontal ganglion, but does possess a white concentrating substance in its brain and frontal ganglion.[3] Thus it would appear that the chromatophorotropic substances which we have found in the nervous system are not materials of general distribution in the nervous tissue of all forms and hence are more likely to have a specific function of a humoral nature within the bodies of those forms in which they occur. The white chromatophores of most crustaceans respond to background changes and all the evidence at hand indicates that they are controlled in this reaction by hormones. Now, as far as we know, the only source of a concentrating principle for this pigment is in the central nervous system, while an antagonistic (dispersing) substance is formed in the sinus gland. A comparable dual control of the black pigment in the telson and uropods of *Crago* is found (Brown and Ederstrom, 1940) in which the sinus gland produces a concentrating substance and part of the central nervous system (commissural ganglia) produces a dispersing substance. We are therefore led to suspect that here in the arthropods the nervous and endocrine mechanisms are more closely associated morphologically than in the higher vertebrates. Some of the hormones are produced by definite glandular bodies apart from the nervous system, while others are produced by cells intimately associated with nerve cells or perhaps possessing the dual function of secretion of hormones and conduction of nerve impulses.

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[2] This effect is apparently not due to acetyl choline since experiments using a wide range of concentrations of this latter substance failed to give similar results.

[3] We have failed to find a red concentrating principle in the annelid nervous system.

The dilution experiments give us no reason for supposing that the red chromatophore-affecting materials from the insect corpora cardiaca and that from the crustacean sinus gland are not chemically the same. The material from the corpora cardiaca loses its effectiveness upon dilution at the same rate as that from the sinus gland. Furthermore, both withstand boiling and both retain their activity for some time in desiccated tissues. If these substances are the same, then a separate substance which disperses white must be present in the sinus gland.

SUMMARY

1. The corpora cardiaca are the organs in the head of the insect which are responsible for the formation of a substance which produces a strong concentration of the pigment of the red chromatophores of crustaceans.

2. The corpora cardiaca still have an effect upon isolated red chromatophores of *Cambarus* when the gland is extracted in 80,000,000 times its volume of Griffeth's solution.

3. Neither the corpora cardiaca nor the corpora allata have any perceptible effect upon the white chromatophores of *Cambarus;* the latter gland is also without effect upon the red chromatophores.

4. The crustacean sinus glands still have an effect upon isolated red chromatophores when extracted in about 14,000,000 times their volume of Griffeth's solution.

5. The sinus glands of crayfishes produce a dispersion of the pigment of the white chromatophores of *Cambarus.*

6. The crustacean sinus gland and the insect corpora cardiaca differ qualitatively from one another with respect to their action upon the white chromatophores. With respect to the red pigment-affecting principle, the two glands are similar in that the rate of loss of effectiveness

with dilution is the same for both, boiling the extracts inactivates neither, and both glands retain their effectiveness for some time in a desiccated state.

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